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(54) Improvements relating to methods and apparatus for measuring spermatozoal motility

(57) A highly collimated light beam 1 is directed onto a sample cell 3 enclosing a sample of sperm solution and reflected therefrom into a microscope 4 and television camera 5. The shape and type of movement of individual spermatozoa is such that the collimated light will only be reflected into the microscope from those spermatozoa which are moving very close to one direction. Images registered by the camera 5 are relayed to an electronic image processor 6 which calculates the rate of movement (and the number, if required) of visible targets moving in one direction between two frames at a known separation, by calculating, e.g. a cross-correlation function for the two line-averaged frames.

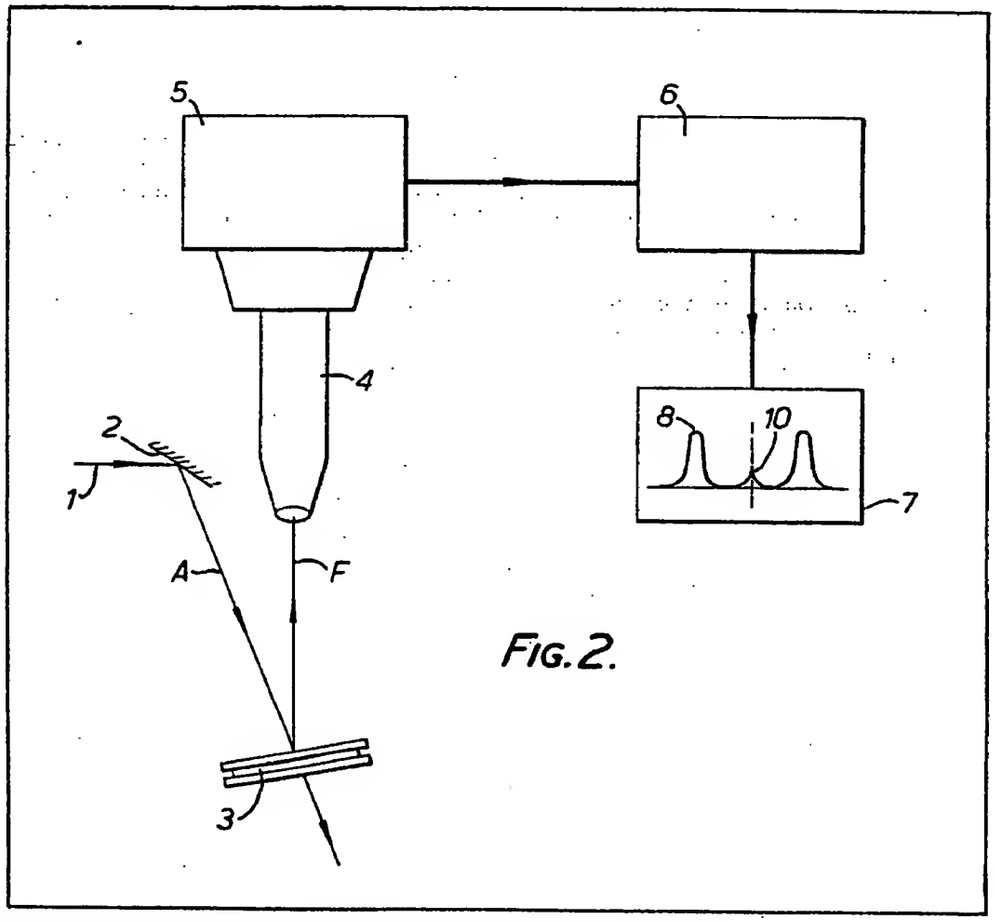

FIG. 2.

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The drawing originally filed was informal and the print here reproduced is taken from a later filed formal copy.

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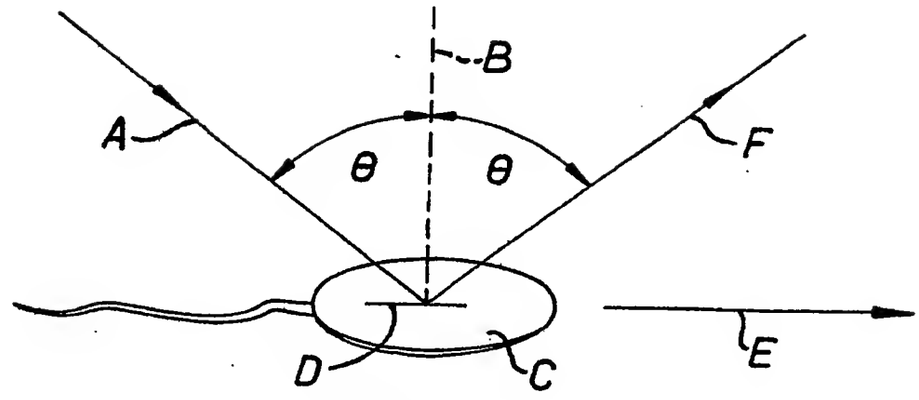

FIG. 1.

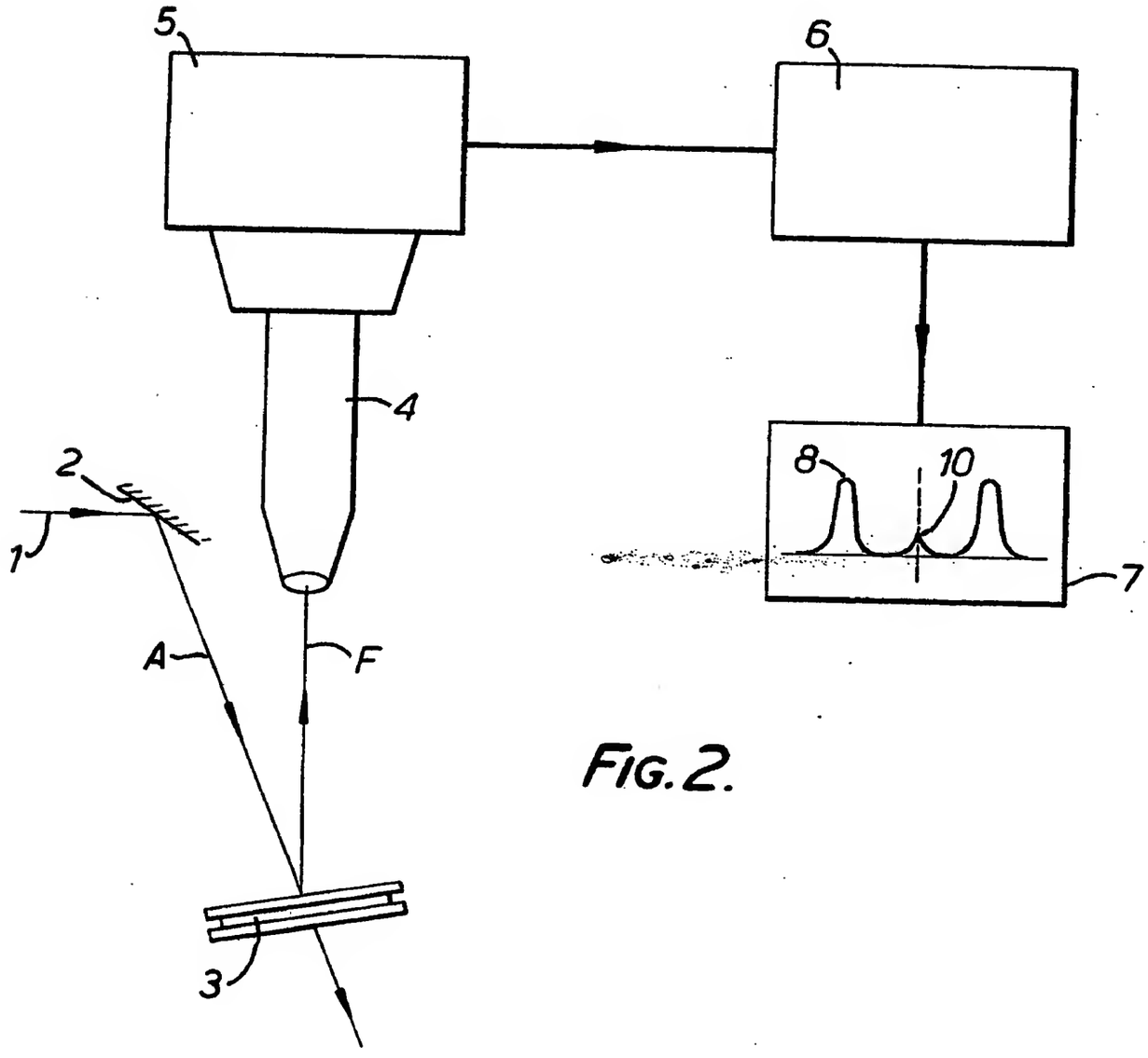

FIG. 2.

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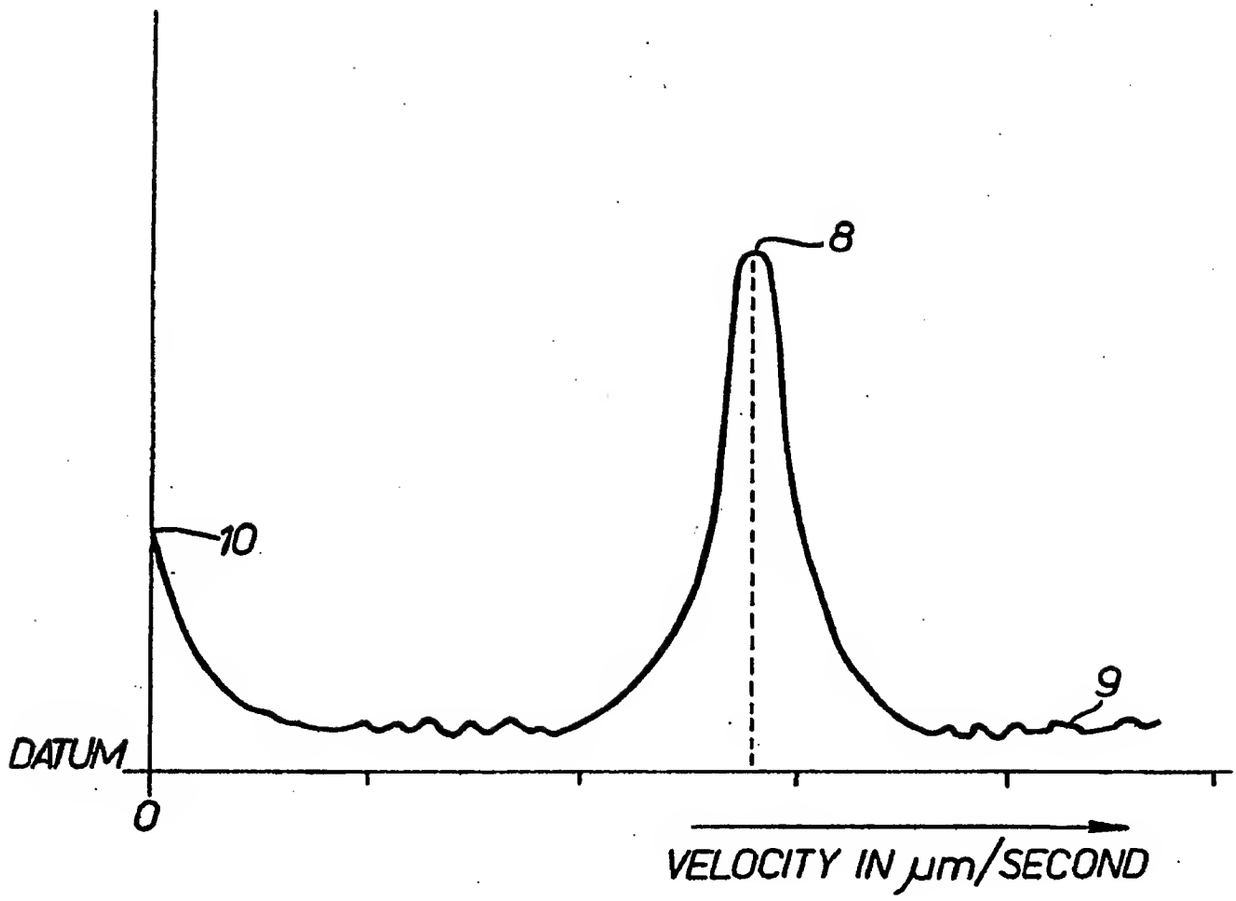

FIG. 3.

SPECIFICATION

Improvements relating to methods and apparatus for measuring spermatozoal motility

This invention relates to a method of and apparatus for measuring spermatozoal motility. In the study of human and animal fertility there arises the need to measure the vigour of motion of spermatozoa, particularly in the practice of artificial insemination, where the viability of samples reconstituted from the deep frozen state requires quantification. The normal practice is to make observations with an optical microscope using skilled operators who may classify the motions on a scale from 1 to 5 of so called "motility". Observations of malformed and irregular swimmers are also of use.

It is an object of this invention to provide apparatus and a method for measuring spermatozoal motility which will operate to provide measurement readings automatically.

From one aspect this invention provides apparatus for measuring spermatozoal activity, comprising a microscope for observing a sample of sperm solution positioned on the microscope stage, a source of a laser or other highly collimated light beam for directing the beam onto the microscope stage to be reflected into the optical axis of the microscope, and a television or other electronic system for recording images produced and processing these images over a period of time to determine a one-dimensional displacement of the images.

The flat ellipsoidal shape of the head of each spermatozoon only reflects the collimated light (within a threshold for recognition of the reflected illumination) when the light beam, flat head and detector are close to a particular specular reflecting position (within about $\pm 3°$). This combined with the helical motion of each organism gives rise to the effect that chaotic flow is converted into an apparent one-dimensional flow. The reflectors have characteristic electrical signatures which act as markers for particular cells which can be applied to an electronic tagging circuit. Ideally the solution will be diluted to give rise to 20 to 30 recorded tracks, but the television equipment could be capable of coping with up to 15 tagged elements per line.

It is preferred that the microscope cell will have a spacing of several hundred microns. Advantageously the microscope stage will be temperature controlled and capable of being tilted. The displacement rate may readily be determined from a cross-correlation of two line averaged frames at a known time separation which could be as low as one frame but more conveniently will be that number of frames during which the mean sperm displacement should be approximately one quarter of the image dimension in the direction of motion being viewed. The apparatus will ideally incorporate display means to show the mean velocity and velocity distribution to the operator.

This apparatus provides means for analysis of the motility of spermatozoa which may be both quantitative and automatic. Electronic counting of identified moving targets and other obvious refinements in combination with the known dilution factor may be employed to give further useful information on live/dead sperm ratio and the number of irregularly moving species. In particular electronic techniques to suppress non-moving species from the inter-frame correlation could be used to improve accuracy.

From another aspect this invention provides a method of measuring spermatozoal motility including the steps of equipping a standard optical microscope with a laser or other highly collimated illumination source, positioning the stage to orient the specimen cells, viewing the images with a television system or other electronic means and processing the electronic image signals, for example by cross-correlation of two frames at a known separation, to determine distance moved and hence velocity.

The invention may be performed in various ways and a preferred embodiment thereof will now be described with reference to the accompanying drawings, in which:—

Figure 1 illustrates the strong scattering geometry of a moving spermatozoon;

Figure 2 is a schematic view of a sperm motility apparatus of this invention; and

Figure 3 is a schematic display of the cross correlation function obtained from the line average of two TV frames using the equipment of Figure 2.

The normal motion of a single organism in a buffer solution which gives a dilution sufficient to avoid mutual interaction is helical, with a pitch approximately equal to the length of the head, which has the shape of a flat ellipsoid. Intensity fluctuation laser scattering systems have been developed which purport to measure sperm velocity by the Doppler shift of the radiation from the moving scatterers. While it is true that the spectrum of the scattered light, as manifest in its fluctuations, varies with the condition of the sample, it is now clear that this simple quantification of motion by the theory of the Doppler effect is not possible due to the complex nature of the targets and their motion. However, it has been noticed recently that the light scattered by a single sperm from a laser beam can be interpreted to a first approximation by considering only that received when the beam, flat head and detector are close to particular reflecting positions to be significant. This configuration is illustrated by way of example only in the accompanying Figure 1. In this case the light beam A, the normal B to the flat orientation of the head C, the long axis D of the head (which is also the swimming direction E) and the scattered light direction F are coplanar. Other positions of strong scattering can occur when the two semi-angles θ differ from each other, but both beams continue to lie in the same plane.

Considering such scattering only, which is very much greater in strength than any other received light, a little geometrical thought shows that with given incident and scattered beam directions, only

those sperm moving very close to a particular planar direction will be observed, namely the plane which contains the projected laser beam and the scattering direction. The physical reason for the high scattering strength under these conditions is provided by calculations of the diffraction of a flattened ellipsiodal object, which has strong lobes in these directions. Viewed under laser or otherwise highly collimated illumination between glass plates the sperm all seem to swim in one direction. Of course, the direction of motion of each individual sperm will be random within the constraints of the specimen cell, being three-dimensionally random in the interior of the cell and two-dimensionally random along the surfaces. In fact, a mechanism of hydrodynamics tends to accumulate the organisms near the walls after a short time and in this case the population in the "observable" direction may be changed by changing the cell wall orientation, the maximum number being seen when a large flat wall is oriented also in the specular plane. By assuming that those sperm in a trajectory to be seen are characteristic of the (invisible) remainder, a simple proportionate analysis of their linear motions will characterise the motions of the sample as a whole.

As shown in Figure 2 a parallel or almost parallel light beam 1 is arranged, for example, by using a small laser and if necessary a mirror 2 to pass through a sample cell 3 which may be oriented so that a large flat face is close to a specular position with respect to the incident beam A and the scattered light direction F that is to say the direction of the optical axis of a microscope 4. It may be preferable to avoid collecting the exactly specular reflections from the cell walls, when sperm moving at a slight angle to the wall rather than parallel to it will then be observed. It may be convenient for viewing to retain the normal microscope illumination at a low level, in which case the subset of sperm in the image which match the strong scattering condition depicted in Figure 1 are picked out spectacularly by flashing in parallel bright lines as they perform their helical progress.

A TV camera 5 relays the images to an electronic image processor 6 which may have a display 7, for example, of the cross correlation coefficients of a pair of separated line averaged TV frames, the line direction being arranged to be the same as that of the motion of the strongly scattering organisms by suitable orientation of either the TV camera or the incident beam azimuth.

Figure 3 shows a typical display where the spatial delay variable of the cross correlation has been calibrated to a velocity using the known time interval between the two frames. The display shows a peak 8 and distribution about it in velocity corresponding to the healthy motile sperm. A background 9 will average to a constant if integrated for a sufficient length of time but in any finite experiment will have fluctuations from incomplete averaging. Depending on the threshold conditions used in the processor to identify an organism, the trace may show a secondary peak 10 at the origin due to a dead or non-motile fraction. The form of the cross correlation function will be duplicated on the opposite side of the origin (as shown in the display 7 in Figure 2) and could usefully be added to improve the statistical accuracy.

The maximum of the correlation coefficient as a function of linear displacement along the direction of the apparent motion gives the modal distance travelled by the sperm in the time delay between the two frames, and hence the corresponding velocity. The spread about this maximum gives the speed distribution. In conjunction with knowledge of the tool organism density, live and dead fractions may be derived. Nutrient additions present in the buffer solutions may be tolerated.

CLAIMS

1. Apparatus for measuring spermatozoal activity, comprising a microscope for observing a sample of sperm solution positioned on the microscope stage, a source of a laser or other highly collimated light beam for directing the beam onto the microscope stage to be reflected into the optical axis of the microscope, and a television or other electronic system for recording images produced and processing these images over a period of time to determine a one-dimensional displacement of the images

2. Apparatus according to claim 1, wherein the microscope cell has a spacing of several hundred microns.

3. Apparatus according to claim 1 or claim 2, wherein the microscope stage is temperature controlled and/or capable of being tilted.

4. Apparatus according to any one of claims 1 to 3, incorporating display means to show the mean velocity and velocity distribution to the operator.

5. Apparatus according to any one of claims 1 to 4, including an electronic counter constructed to identify and count moving targets.

6. Apparatus according to any one of claims 1 to 5, including electronic means for suppressing non-moving species from the inter-frame correlation.

7. Apparatus according to claim 1 and substantially as herein described.

8. A method of measuring spermatozoal motility including the steps of equipping a standard optical microscope with a laser or other highly collimated illumination source, positioning the stage to orient the specimen cells, viewing the images with a television system or other electronic means and processing the electronic image signals to determine distance moved and hence velocity.

9. A method according to claim 8, wherein the electronic image signals are determined by cross-correlation of two frames at a known separation.

10. A method according to claim 9, wherein the number of frames between cross-correlations is

that during which the mean sperm displacement is approximately one quarter of the image dimension in the direction of motion being viewed.

11. A method according to claim 8 and substantially as herein described with reference to the accompanying drawings.

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PN - SU934383 A 19820607
PD - 1982-06-07
PR - SU19802990320 19801009
OPD - 1980-10-09
TI - DEVICE FOR DETERMINING SPEEDS OF OBJECTS AT DETERMINING OF CHEMICAL COMPOUND ACTIVITY
IN - SKIBENKO VASILIJ V
PA - NII BIOLOG ISPYTANIYAM KHIM SO (SU)

TI - Speed measuring system esp. for biological objects - has preparation and feed units to permit analysis of motion of cells subjected to different concn. of chemical compounds
PR - SU19802990320 19801009
PN - SU934383 B 19820607 DW198316 004pp
PA - (CHEM-R) CHEMICAL BOND RES
IC - G01P3/48 ;H04N7/18
IN - SKIBENKO V V
AB - SU-934383 The system is based on Parent Spec. and has the additional facility of analysis of different types of cell motion, e.g. Brownian movement, spontaneous displacement and movement towards a phagocytosis object (Chemotaxis) in the case of neutrophils.
- Groups of cells are sent by a feeder (11) to a preparation unit (10) where they are dosed with given concs. of chemical compounds in prepared solns.. (9). An undosed control cell accompanies the test cells.
- A t.v. camera (1), an exposure unit (3) and an exposure sensor (4) produce video signals which are fed to an area measuring unit (5) which measures total cell area and a cell counter (6).
- After a given time interval the process is repeated to obtain a new area value. The mean speed of movement of the cells is calculated in an arithmetic unit (7) as the difference between the 2 areas divided by the root of the 1st area all times the root of pi divided by the product of the time interval and the number of cells. The speed values are compared with that of the control cell by a comparator (12) and the differences are fed to a display unit indicating the activity of the compounds during action on cells whose speed of movements varies with the concentration of the compound.
Bul.21/7.6.82 (4pp Dwg.No.1/1)
OPD - 1980-10-09
AN - 1983-F3166K [16]